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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Patrick D.	Kane	Anchorage, Alaska

[ ] *Additional Inventors are being named on the ____ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (280 characters max)

LOCALIZED NON-INVASIVE BIOLOGICAL MODULATION SYSTEM

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ENCLOSED APPLICATION PARTS *(check all that apply)*

[X] Specification *Number of Pages* 18 — [ ] Small Entity Statement

[X] Drawing(s) *Number of Sheets* 7 — [X] Other (specify) 1 page of abstract; applicant is a small entity

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Respectfully submitted,

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Date 9/28/01

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Docket Number: 019028/51940-P

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

Practi[redacted]r's Docket No. 019028/51940-P *PATENT*

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Kane, Patrick D.
Application No.: Unassigned Group No.: Unassigned
Filed: Herewith Examiner: Unassigned
For: LOCALIZED NON-INVASIVE BIOLOGICAL MODULATION SYSTEM

Assistant Commissioner for Patents
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LOCALIZED NON-INVASIVE BIOLOGICAL MODULATION SYSTEM

FIELD OF THE INVENTION

[001] The present application is directed to methods for localized non-invasive delivery of biological modulating agents throughout the body, particularly for the delivery of neuromodulators to specific sites within the brain.

BACKGROUND OF THE INVENTION

[002] There are a range of methods of delivery of an agent to a subject. For *in vivo* administration, methods include catheters, injection, scarification, etc. For example, stereotaxic injection can be used to direct delivery of an agent to a desired location in the brain. Stereotaxic surgery is performed using standard neurosurgical procedures [Pellegrino and Clapp, *Physiol. Behav.* 7: 863-8 (1971)]. Additionally, agents can be delivered by intracerebroventricular ("icv") infusion using a minipump infusion system, such as a SynchroMed Infusion System. A recent method based on bulk flow, termed convection, has also proven effective at delivering large molecules to extended areas of the brain and may be useful in delivering the viral particle to the target cell [R. Bobo et al., *Proc. Natl. Acad. Sci. USA* 91: 2076-80 (1994); P. Morrison et al., *Am. J. Physiol.* 266: R292-305 (1994)]. Other methods can be used including catheters, intravenous,

p[redacted]teral, intraperitoneal and subcutaneous injection, oral or other known routes of administration.

[003] However, many of these methods are systemic, or at best regional in application. This can result in delivery of an agent to normal tissues, where the effect of the agent can be deleterious. Thus, a method for targeted delivery of an agent to only a particular region would be desirable. It would also be desirable to do this in as non-invasive a manner as possible. Accordingly, localized targeted drug delivery is highly desirable for a wide array of applications. For example, the function of the central nervous system relies on the interconnectivity of specific subsets of neurons, which communicate using many different neurotransmitters. Many neurodegenerative diseases are characterized by loss of function of these connections, known as synapses. For example, Parkinson's Disease is a loss of dopaminergic activity in the pigmented neurons of the substantia nigra. Thus, it would be highly desirable to be able to deliver agents including drugs, genes, etc. in a non-invasive manner to a very specific site.

[004] The brain presents particular needs and challenges for targeted drug delivery.

[005] For example, the ability to excite or inhibit the activity of specific subsets of neurons in specific regions of the brain. The inability of many agents to cross the blood-brain barrier also causes problems.

[006] While sophisticated techniques for drug delivery have been developed, there remains a need for improved methods for the precise localized deposition of biologically active agents. Many existing methods rely on invasive techniques, such as localized injection to deliver an agent to its site of action. Even then the agent may disperse from that site. Moreover, such techniques are inherently fraught with the risks of infection

associated with any invasive procedure. Furthermore, certain tissues, such as the brain, are particularly sensitive to any intervention. Thus, it would be highly desirable to have a non-invasive method for the localized delivery of agents.

[007] One advance on drug delivery has been the development of liposomes, including time-release liposomes.

[008] Liposomes consist of at least one lipid bilayer membrane enclosing an aqueous internal compartment. Conventional liposomes are formulated to carry therapeutic agents, drugs or other active agents either contained within the aqueous interior space (water soluble active agents) or partitioned into the lipid bilayer (water-insoluble active agents). Active agents that have short half-lives in the bloodstream are particularly suited to delivery via liposomes. Many anti-neoplastic agents, for example, are known to have a short half-life in the bloodstream such that their parenteral use is not feasible. However, the use of liposomes for site-specific delivery of active agents via the bloodstream is limited by the rapid clearance of liposomes from the blood by cells of reticuloendothelial system (RES).

[009] Liposomes are normally not leaky but will become so if a hole occurs in the liposome membrane, if the membrane degrades or dissolves, or if the membrane temperature is increased to the phase transition temperature. The elevation of temperature (hyperthermia) at a target site in a subject to raise liposome temperature above the phase transition temperature, and thereby cause the release of the liposome contents, has been used for the selective delivery of therapeutic agents. Yatvin et al., Science 204:188 (1979). Recently liposome formulations capable of delivering

therapeutic amounts of active agents in response to mild hyperthermic conditions (U.S. Patent No. 6,200,598).

[0010] Thermosensitive liposomes have been developed which retain their structure at 37°C, human body temperature, but are destroyed at even slightly elevated temperatures (e.g. 42°C). Microwaves have been used for localized drug delivery by spatial localized destruction of thermosensitive liposomes (for example to treat tumors in the hand). However, microwaves do not offer a high degree of localization. Thus, in situations where precise control is desired, for example when targeting specific regions of the brain, it is not satisfactory. Thermosensitive liposomes have also been used with an invasive source of heat for localized drug delivery. However, as described above, such invasive techniques are associated with infection risks and are not available for all regions of the body.

[0011] Thus, there remains a need for improved methods of localized drug delivery. In particular, it would be highly desirable to have a non-invasive method for the localized delivery of biologically active molecules.

SUMMARY OF THE INVENTION

[0012] The present invention provides methods for non-invasive localized delivery of biologically active molecules, comprising packaging a molecule(s) of interest inside an energy sensitive vehicle, such as a thermosensitive vesicle, administering said vesicles to a subject, and inducing localized release of said molecules from said vesicles using a focused energy source. The thermosensitive vesicles include thermosensitive liposomes

ar[redacted]ermosensitive polymer nanovesicles. The vesicles may be delivered to a subject by any technique, including infusion. The molecules may be released from the vesicles using any non-invasive method which induces localized hypothermia, including focused ultrasound.

[0013] One preferred embodiment of the present invention provides methods for treating neural conditions, using antibody-conjugated immunoliposomes which cross the blood-brain barrier to deliver neuromodulators to the brain. Neuromodulators include molecules which activate or inhibit specific populations of neurons. Preferred neural conditions include epilepsy, Alzheimer's disease, Parkinson's disease, stroke, developmental learning disabilities, and post-traumatic neuronal cell loss.

[0014] Other preferred embodiments of the present invention provides methods for treating arthritis.

[0015] Another embodiment of the present invention provides a method for targeted adipose tissue destruction.

[0016] One preferred embodiment of the present invention provides a method for targeted gene therapy.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] Figure 1 is a depiction of the formation of liposomes by the dispersion of lipid molecules in water, including the entrapment of drugs, antibodies, proteins, and peptides.

[0018] Figure 2 is a detailed depiction of a liposome.

[0019] Figure 3 depicts a standard procedure for preparing liposomes containing drugs (in either the lipid bilayer or inside the vesicle).

[0020] Figure 4 depicts Focused Ultrasound (FUS) methods, including their application to a subject's head using phased-array transducers.

[0021] Figure 5 depicts the use of magnetoliposomes for vesicle localization.

[0022] Figure 6 depicts the release of a neurotransmitter during the destruction of a liposome (for example, the destruction of a thermosensitive liposome in the presence of increased temperature).

[0023] Figure 7 depicts non-invasive neuronal modulation.

DETAILED DESCRIPTION OF THE INVENTION

[0024] We have now discovered a method for localized non-invasive delivery of biologically active molecules, comprising packaging a molecule of interest inside an energy-sensitive vesicle, preferably a thermosensitive vesicle, administering said vesicles to a subject, and inducing localized release of said molecules from said vesicles using a focused energy source.

Thermosensitive vesicles

[0025] Any thermosensitive vesicle which can package a molecule of interest and which is intact at body temperature (i.e. 37° C) but destroyed at any other, non-body temperature which can be tolerated by a subject may be used. Examples of thermosensitive vesicles include thermosensitive liposomes and thermosensitive polymer nanovesicles.

[0026] Thermosensitive liposomes are known in the art. Liposomes according to the present invention may be prepared by any of a variety of techniques that are known in the art. See, e.g., U.S. Pat. No. 4,235,871; Published PCT applications WO 96/14057; New RRC, Liposomes: A practical approach, IRL Press, Oxford (1990), pages 33-104; Lasic D D, Liposomes from physics to applications, Elsevier Science Publishers, Amsterdam, 1993; Liposomes, Marcel Dekker, Inc., New York (1983). Entrapment of an active agent within liposomes of the present invention may also be carried out using any conventional method in the art. In preparing liposome compositions of the present invention, stabilizers such as antioxidants and other additives may be used as long as they do not interfere with the purpose of the invention. Examples include co-polymers of N-isopropylacrylamide (*Bioconjug. Chem.* 10:412-8 (1999)).

[0027] A method of preparing a liposomal formulation according to the present invention comprises mixing the bilayer components in the appropriate proportions in a suitable organic solvent, as is known in the art. The solvent is then evaporated to form a dried lipid film. The film is rehydrated (at temperatures above the phase transition temperature of the lipid mixture) using an aqueous solution containing an equilibrating amount of the surface active agent and a desired active agent. The liposomes formed after rehydration can be extruded to form liposomes of a desired size, as is known in the art. For example, where liposomes composed of 80:20 DPPC:MPPC are produced, rehydration is carried out at a temperature above the phase transition temperature of this particular lipid mixture (above 39.degree.C.). The aqueous solution used to rehydrate the lipid film comprises an equilibrating amount of lysolipid monomers (e.g., a concentration equal to the Critical Micelle Concentration of MPPC).

[0028] Polyethylene glycol (PEG) may be incorporated into the liposome bilayer to inhibit fusion with undesired membranes (Bulte et al., *Proc. Intl. Soc. Mag. Reson. Med.*, Fifth Annual Meeting, p. 1596 (1997)).

[0029] The thermosensitive vesicle may include any other useful molecules. For example, the vesicle may include a monoclonal antibody on its surface which allows targeting of the vesicle to a desired site. For example, an antibody to the transferrin receptor, which can cross the blood-brain barrier, may be used to target vesicles to the brain. Similarly, membrane-colloidal magnetite (Fe304) may be incorporated into the liposome bilayer; the application of a magnetic field may then be used to localize the vesicles to a desired site (Bulte et al., *Proc. Soc. Mag. Reson.*, Third Annual Meeting, p. 1139 (1995)).

[0030] The thermosensitive vesicles may be administered to a subject using known means. For example, injection.

Focused energy sources

[0031] Any focused energy source, preferably a heat source capable of inducing highly localized hyperthermia to promote the destruction of the thermosensitive vesicles may be used. For example, focused ultrasound.

Active Agents

[0032] As used herein, an active agent "in the interior" or "entrapped within" the liposome is that which contained in the interior space of the liposome, compared to that partitioned into the lipid bilayer and contained within the vesicle membrane itself. As used herein, an active agent "within" or "entrapped within" the lipid bilayer of a liposome

carried as a part of the lipid bilayer, as opposed to being contained in the interior space of the liposome. Active agents may be in any form suitable for use in liposomes, as is known in the art, including but not limited to aqueous solutions of active agents. Aqueous solutions of active agents within liposomes of the present invention may be at the same osmotic pressure as that of the body fluid of the intended subject, or at an increased osmotic pressure (see U.S. Pat. No. 5,094,854); the aqueous solutions may also contain some precipitated active agent, as is known in the art. A preferred active agent for encapsulation in the interior of the liposome is any water soluble, weak base agent.

[0033] The incorporation of certain active agents (such as some anesthetics) in liposomes of the present invention may additionally alter (enhance or inhibit) the release of contents from the liposome, or alter the transition temperature of the liposome, compared to that which would be seen in a similar liposome that did not contain the active agent.

[0034] The incorporation of certain active agents (such as some anesthetics) in liposomes of the present invention may additionally alter (enhance or inhibit) the release of contents from the liposome, or alter the transition temperature of the liposome, compared to that which would be seen in a similar liposome that did not contain the active agent.

[0035] The administration of antineoplastic or antitumor drugs such as doxorubicin, cisplatin and methotrexate using thermosensitive liposomes in combination with hyperthermia at the desired target site has been reported. See, e.g., Magin and Weinstein In: Liposome Technology, Vol. 3, (Gregoriadis, G., ed.) p. 137, CRC Press, Boca Raton, Fla. (1993); Gaber et al., Intl. J. Radiation Oncology, Biol. Physics, 36(5):1177 (1996).

[0036] Active agents suitable for use in the present invention include therapeutic drugs and pharmacologically active agents, nutritional molecules, cosmetic agents, diagnostic agents and contrast agents for imaging. As used herein, active agent includes pharmacologically acceptable salts of active agents. Suitable therapeutic agents include, for example, antineoplastics, antitumor agents, antibiotics, antifungals, anti-inflammatory agents, immunosuppressive agents, anti-infective agents, antivirals, anthelminthic, and antiparasitic compounds. Methods of preparing lipophilic drug derivatives which are suitable for liposome formulation are known in the art (see e.g., U.S. Pat. No. 5,534,499 to Ansell, describing covalent attachment of therapeutic agents to a fatty acid chain of a phospholipid).

[0037] In treating tumors or neoplastic growths, suitable compounds may include anthracycline antibiotics (such as doxorubicin, daunorubicin, carinomycin, N-acetyladriamycin, rubidazone, 5-imidodaunomycin, N30 acetyldaunomycin, and epirubicin) and plant alkaloids (such as vincristine, vinblastine, etoposide, ellipticine and camptothecin). Other suitable agents include paclitaxel (TAXOL.RTM.; a diterpenes isolated from the bark of the yew tree and representative of a new class of therapeutic agents having a taxane ring structure) and docetaxol (taxotere); mitotane, cisplatin, and phenesterine.

[0038] Anti-inflammatory therapeutic agents suitable for use in the present invention include steroids and non-steroidal anti-inflammatory compounds, such as prednisone, methyl-prednisolone, paramethazone, 11-fludrocortisol, triamciniolone, betamethasone and dexamethasone, ibuprofen, piroxicam, beclomethasone; methotrexate, azaribine, etretinate, anthralin, psoralins; salicylates such as aspirin; and immunosuppresant agents

such as cyclosporine. Antiinflammatory corticosteroids and the antiinflammatory and immunosuppressive agent cyclosporine are both highly lipophilic and are suited for use in the present invention.

[0039] Additional pharmacologic agents suitable for use in liposomes of the present invention include anesthetics (such as methoxyflurane, isoflurane, enflurane, halothane, and benzocaine); antiulceratives(such as cimetidine); antiseizure medications such as barbituates; azothioprine (an immunosuppressant and antirheumatic agent); and muscle relaxants (such as dantrolene and diazepam).

[0040] Imaging agents suitable for use in the present liposome preparations include ultrasound contrast agents, radiocontrast agents (such as radioisotopes or compounds containing radioisotopes, including iodo-octanes, halocarbons, and renograf in), or magnetic contrast agents (such as paramagnetic compounds).

[0041] Nutritional agents suitable for incorporation into liposomes of the present invention include flavoring compounds (e.g., citral, xylitol), amino acids, sugars, proteins, carbohydrates, vitamins and fat. Combinations of nutritional agents are also suitable.

Administration and Vesicle Size

[0042] Vesicles including polymer nano vesicles and liposomes of the present invention may be administered using methods that are known to those skilled in the art, including but not limited to delivery into the bloodstream of a subject or subcutaneous administration of the vesicle. For example, the liposomes may be administered by any suitable means that results in delivery of the liposomes to the treatment site. It does not matter if the vesicle also goes to other sites because the agent will only be released where

energy source is directed. For example, liposomes may be administered intravenously and thereby brought to the treatment site by the normal blood flow; it is the precise heating of the targeted site that results in the liposomal membranes being heated to the phase transition temperature so that the liposomal contents are preferentially released only at the site of the tumor.

[0043] Where treatment of a tumor or neoplasm is desired, effective delivery of a liposome-encapsulated active agent via the bloodstream requires that the liposome be able to penetrate the continuous (but "leaky") endothelial layer and underlying basement membrane surrounding the vessels supplying blood to a tumor. Liposomes of smaller sizes have been found to be more effective at extravasation into tumors through the endothelial cell barrier and underlying basement membrane which separates a capillary from tumor cells. See, e.g., U.S. Pat. No. 5,213,804 to Martin et al.

[0044] As used herein, "solid tumors" are those growing in an anatomical site other than the bloodstream (in contrast to blood-borne tumors such as leukemias) Solid tumors require the formation of small blood vessels and capillaries to nourish the growing tumor tissue.

[0045] It will further be appreciated that the vesicles of the present invention may be utilized to deliver of anti-infective agents to sites of infection, via the bloodstream. The use of for example, liposomes containing a vesicle-forming lipid derivatized with a hydrophilic polymer, and having sizes ranging between 0.07 and 0.2 microns, to deliver therapeutic agents to sites of infection is described in published PCT patent application WO 93/19738. In accordance with the present invention, the anti-infective agent of choice is entrapped within a liposome having a membrane according to the present

ention, and the resulting liposomal formulation is administered parenterally to a subject, preferably by intravenous administration. If desired, localized hyperthermia may be induced at the site of infection to cause the preferential release of liposomal contents at that site.

[0046] The size of vesicles in a preparation will depend upon the active agent contained therein and/or the intended target. Liposomes or new vesicles of between 0.05 to 0.3 microns in diameter are suitable for tumor administration (U.S. Pat. No. 5,527,528 to Allen et al.) Sizing of vesicles according to the present invention may be carried out according to methods known in the art, and taking into account the active agent contained therein and the effects desired (see, e.g., U.S. Pat. No. 5,225,212 to Martin et al; U.S. Pat. No. 5,527,528 to Allen et al). A preferred embodiment of the present invention is a vesicle of less than 10 microns in diameter, or a vesicle preparation containing a plurality e.g., liposomes of less than 10 microns in diameter. In a further preferred embodiment of the present invention, vesicles are from about 0.05 microns or about 0.1 microns in diameter, to about 0.3 microns or about 0.4 microns in diameter. Vesicle preparations may contain vesicles of different sizes.

[0047] In another preferred embodiment of the present invention, vesicles are from about 50 nm, 100 nm, 120 nm, 130 nm, 140 nm or 150 nm, up to about 175 nm, 180 nm, 200 nm, 250 nm, 300 nm, 350 nm, 400 nm or 500 nm in diameter.

[0048] In one aspect of the present invention, the vesicles are prepared to have substantially homogeneous sizes in a selected size range. One effective sizing method involves extruding an aqueous suspension of the vesicles through a series of polycarbonate membranes having a selected uniform pore size; the pore size of the

membrane will correspond roughly with the largest sizes of liposomes produced by extrusion through that membrane. See e.g., U.S. Pat. No. 4,737,323 (Apr. 12, 1988).

[0049] In a further aspect of the present invention, vesicles are dispersed in physiological saline or PBS to provide an aqueous preparation of vesicles. For example the aqueous preparation may further include an equilibrating amount of the surface active agent contained in the liposome bilayer, to reduce or prevent loss of the surface active agent from the liposome bilayer into solution. Liposomes composed of DPPC:MPPC may be contained in physiological saline or PBS that contains from about 1 mmM to about 5 mM of MPPC monomer.

[0050] The amount of active agent to be entrapped within or carried by liposomes according to the present invention will vary depending on the therapeutic dose and the unit dose of the active agent, as will be apparent to one skilled in the art. In general, however, the preparation of vesicles of the present invention is designed so the the largest amount of active agent possible is carried by the vesicle. Vesicles of the present invention may be of any type, however, LUVs are particularly preferred.

Applications

[0051] The method of the present invention may be used for the localized delivery of a wide variety of agents to treat a wide variety of conditions. For example, the delivery of neuromodulators to specific regions of the brain may be used to modulate neuronal transmission, including the delivery of inhibitory neurotransmitters to treat seizure foci in epileptics; excitatory neurotransmitters to treat Alzheimer's patients; excitatory neurotransmitters to enhance dopaminergic activity in Parkinson's patients; inhibitory neurotransmitters (such as NMDA antogonists) to prevent brain damage in stroke

vi[redacted]ms, including emergency stroke treatment; agents to treat developmental learning disabilities (such as ADHD); and neurotransmitters to prevent post-traumatic neuronal cell loss. The method of the present invention may also be used to deliver anti-arthritic agents such as anti-inflammatory drugs to sites of athritic lesions in arthritis patients. Another embodiment prevents localized deposition of agents to treat artherosclerotic lesions. In another embodiment, the present method may be used to deliver cytotoxic agents for localized tissue destruction, including for example solid tumors as well as undesired adipose tissue. A further embodiment of the present invention provides the localized delivery of nucleic acids for targeted gene therapy.

EXAMPLES

Epilepsy (rodent)

Subject animal implanted with seizure inducing substance and recording device
Subject injected with vesicle packaged inhibitory neurotransmitter
Immediately following seizure instigation, stimulation electrodes deactivated
Seizure propagation monitored by surface EEG
tFUS activated and focused on seizure foci
Inhibitory neurotransmitter released at seizure foci and epileptiform activity subdued
Inhibitory neurotransmitter release is continued and slowly reduced, inducing LTD and extinguishing the chance of epileptic relapse

Alzheimer's (rodent)

Subject animal bred with Alzheimer's dementia mutation
Control, non-Alzheimer's, non-tFUS animal run through memory task
Alzheimer's animal run through an identical memory task, demonstrating diminished task completion ability
Subject injected with vesicle packaged excitatory neurotransmitter
tFUS targeted to hippocampal region of Alzheimer's subject
Synthetic θ–rhythm induced in hippocampal formation of Alzheimer's subject, replacing function of deteriorated septal cholinergic cells, and enhancing memory retention
Alzheimer's subject run through memory task under tFUS influence, demonstrating normal to exemplary task completion ability

Parkinson's (rodent)

Subject animal bred with Parkinson's mutation
Control, non-Parkinson's, non-tFUS animal run through a motor function related task
Parkinson's animal run through an identical memory task, demonstrating diminished task completion ability
Subject injected with vesicle packaged excitatory neurotransmitter
tFUS targeted to substantia nigra (pars compacta) of Parkinson's subject, enhancing dopaminergic activity, and demonstrating normal task completion ability
tFUS also focused on pathways utilized by aforementioned region to modulate other areas of the basal ganglia and premotor cortex, further alleviating the tremor and inability to initiate movement prevalent in Parkinson's patients

Stroke (rodent)

Local population of subject animal neurons damaged due to lack of blood flow to region.
Target release of inhibitory neurotransmitter surrounding damaged area prevents disabling brain damage.

Emergency stroke treatment:
Though initial damage caused by strokes is due mainly to lack of oxygen to cells, this usually only causes permanent damage to a small amount of cells. The main damage is due to the subsequent apoptosis and surrounding cell death caused by excessive depolarization and calcium influx. Depositing local modulators (e.g. NMDA antagonist) would prevent brain damage to critical brain regions while allowing the patient to sustain activity in neural systems controlling heart function, breathing, and the like while s/he heals.

DLD (rodent)

A.
Subject animal with DLD (i.e. ADHD) is monitored for hyperactivity during behavioral task
Control subject is injected with ADHD medication and monitored for side effects
Test subject injected with vesicle packaged ADHD medication
tFUS targeted to area relevant to ADHD cause
Medication released only in necessary areas, reducing and eliminating adverse side effects common to ADHD medication use
Subject run through behavioral task under and after UEDP treatment , demonstrating hyperactivity extinction

B.
Subject animal with DLD (i.e. ADHD) is monitored for hyperactivity during behavioral task

Control subject is injected with ADHD medication and monitored for side effects
Test subject injected with vesicle packaged neurotransmitter
tFUS targeted to areas relevant to ADHD cause
Excitability and activity modulated in relevant areas
Subject run through behavioral task under and after UEDP treatment , demonstrating hyperactivity extinction

Post-Traumatic Neuronal Cell Loss (rodent)

Subject animal with Neurotoxicity monitored during behavioral task
Subject injected with vesicle packaged neurotransmitter
tFUS targeted to damaged areas
Excitability and activity manipulated to reestablish normal plasticity in affected brain regions
Subject run through behavioral task, demonstrating reestablished brain function

hLTP (primate)

Subject run through memory task; completion capability recorded
Subject injected with vesicle packaged excitatory neurotransmitter
tFUS targeted to hippocampus
θ–rhythm oscillation modulated, reinforcing septal inputs and allowing control over stimuli retention
Subject run through memory task under tFUS influence, demonstrating increased stimuli retention, associational ability, and learning speed

hLTD (primate)

A.
Subject run through memory task; completion capability recorded
Subject injected with vesicle packaged inhibitory neurotransmitter
tFUS targeted to hippocampus
Subject run through different memory task (of same difficulty) under LTD inducing tFUS influence, demonstrating diminished stimuli retention, and temporary loss of learning ability

B.

Subject run through multi-trial memory task; increasing completion speed recorded
Subject injected with vesicle packaged inhibitory neurotransmitter
tFUS targeted to hippocampus; regional LTD induced
Subject run through same memory task; completion capability recorded, demonstrating loss of previously gained memory

NOTE: Though initial experimentation is confined to the hippocampus, plasticity modulation is not limited to any single brain region. In fact, expanding hLTP/hLTD manipulation to other regions will enhance desired effects, and is a natural next step.

Arthritis (rodent)

Subject animal with arthritis injected with vesicle packaged, arthritis-killing drug
tFUS targeted to arthritis location
High potency drug selectively released , demonstrating arthritis destruction with insignificant or zero damage to surrounding cells

NOTE: Though arthritis is destroyed in the above experiment, this procedure is not limited to a particular condition. Any spatially localized disease can be annihilated by this drug delivery method (e.g. cancer).

Targeted Fat Cell Destruction (rodent)

Overweight subject is injected with vesicle packaged, fat-cell-killing compound
tFUS is targeted to unwanted fat excess, demonstrating localized fat annihilation

All references described herein are incorporated herein by reference.

ABSTRACT

The present invention provides methods for non-invasive localized delivery of biologically active molecules, comprising packaging a molecule(s) of interest inside a thermosensitive vesicle, administering said vesicles to a subject, and inducing localized release of said molecules from said vesicles using a focused heat source. The thermosensitive vesicles may be thermosensitive liposomes or thermosensitive polymer nanovesicles. The vesicles may be delivered to a subject by any technique, including infusion. The molecules may be released from the vesicles using any method which induces localized hypothermia, including focused ultrasound.

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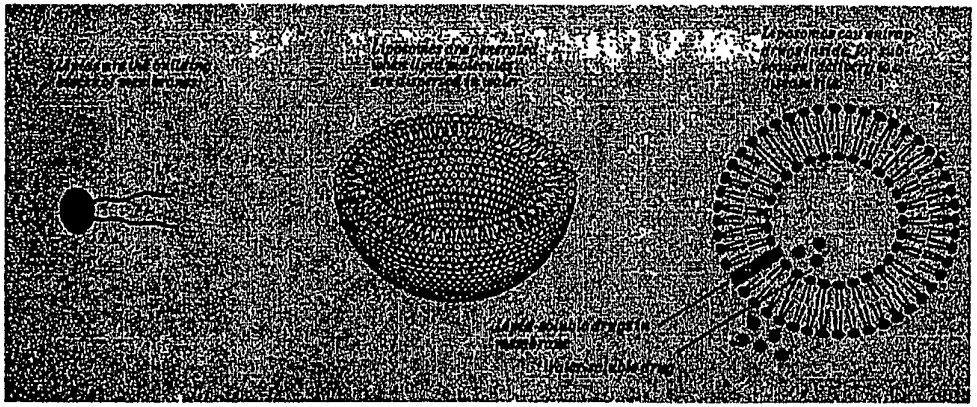
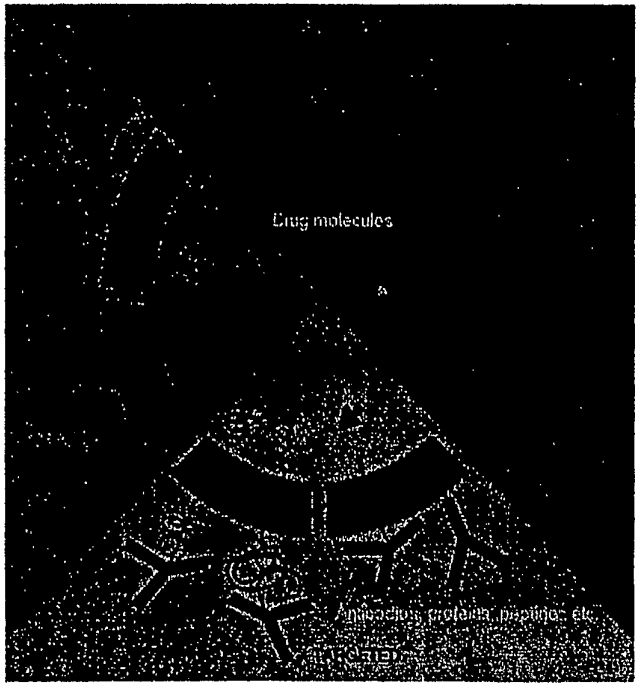
Liposome





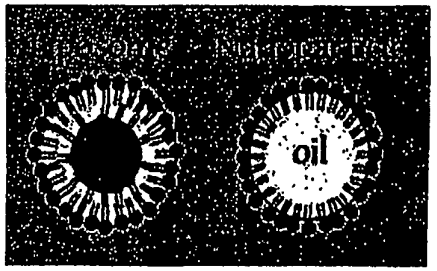


Figure 1

Liposome Package

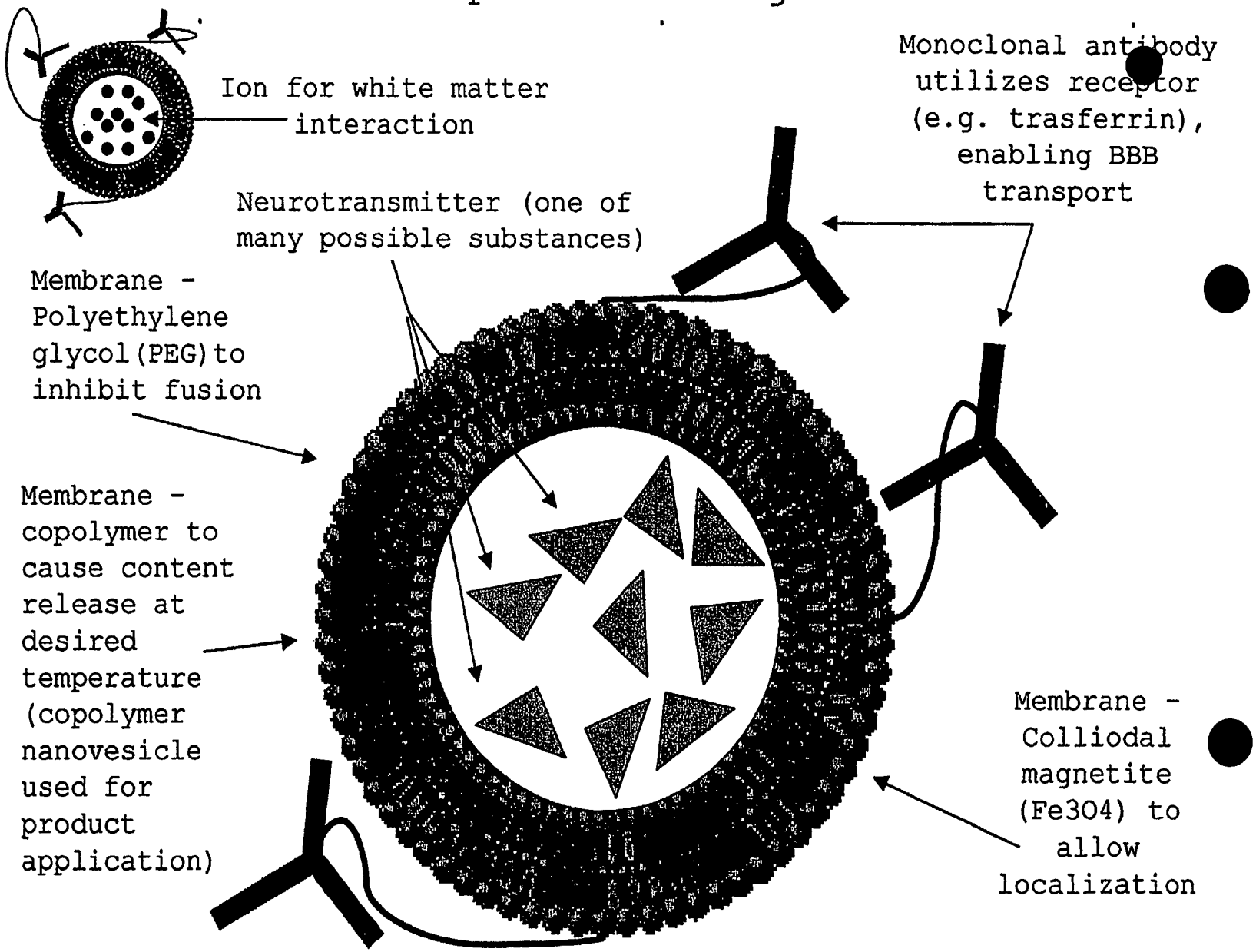


Figure 2

LIPOSOME PREPARATION

Standard Procedure

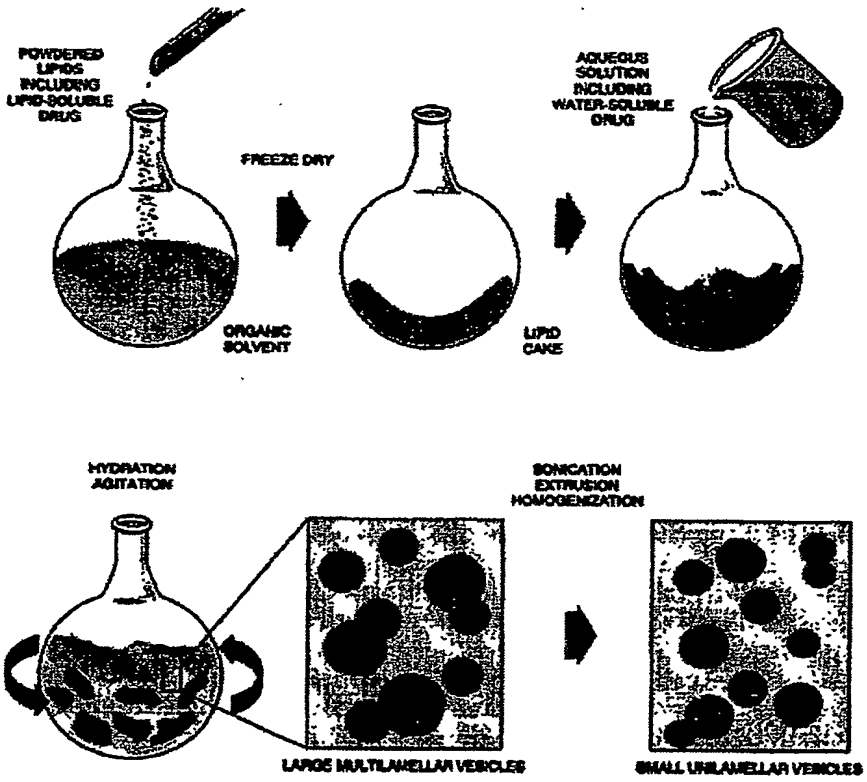
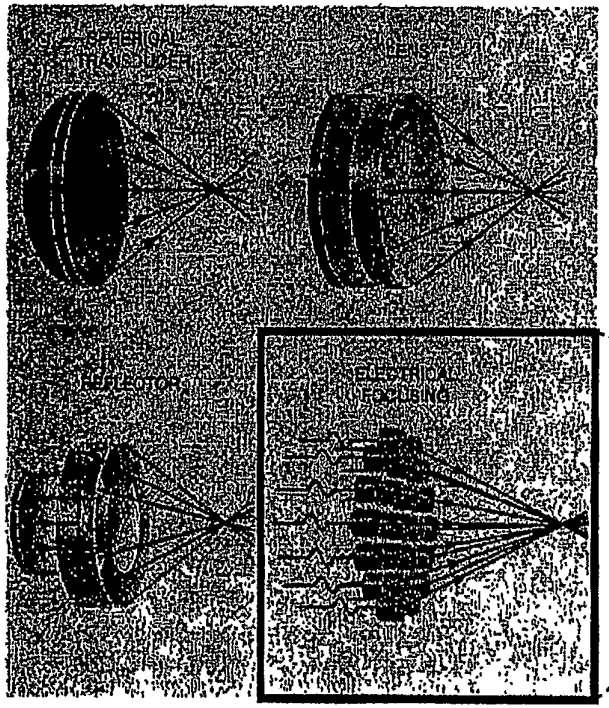


Figure 3

Controlled Heat Deposition

Focused Ultrasound (FUS)
Methods



Phased-array
Transducers

Surface EEG

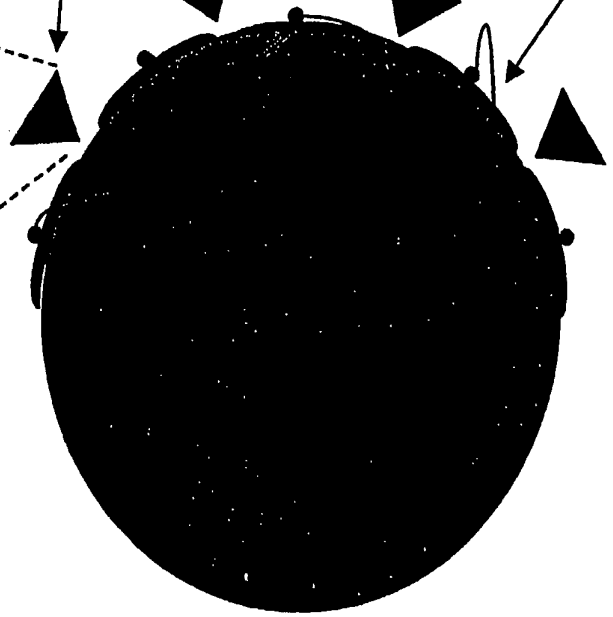


Figure 4

Liposome Package Localization

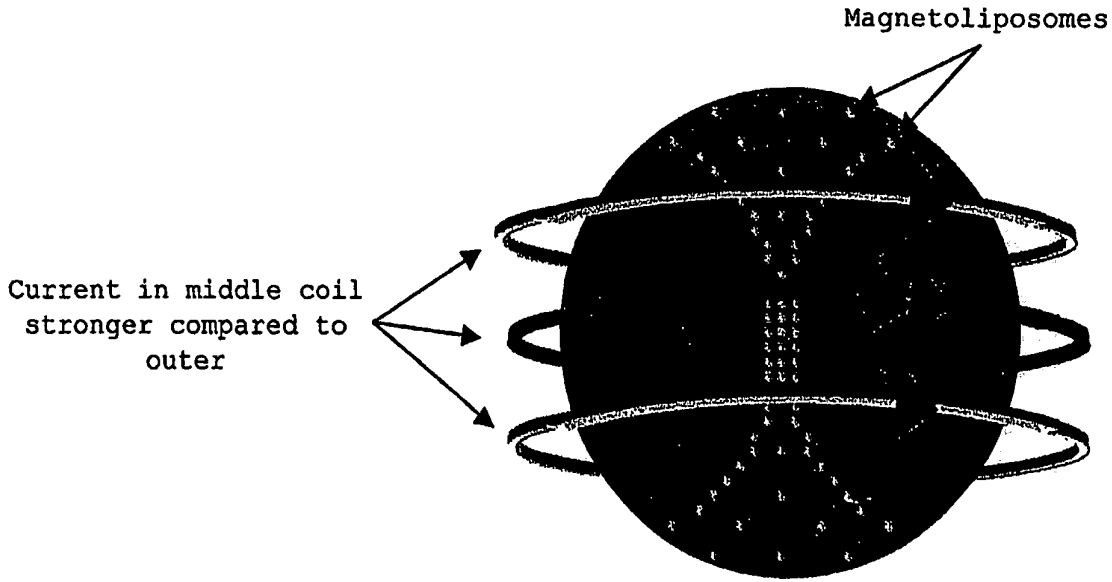


Figure 5

Package Destruction

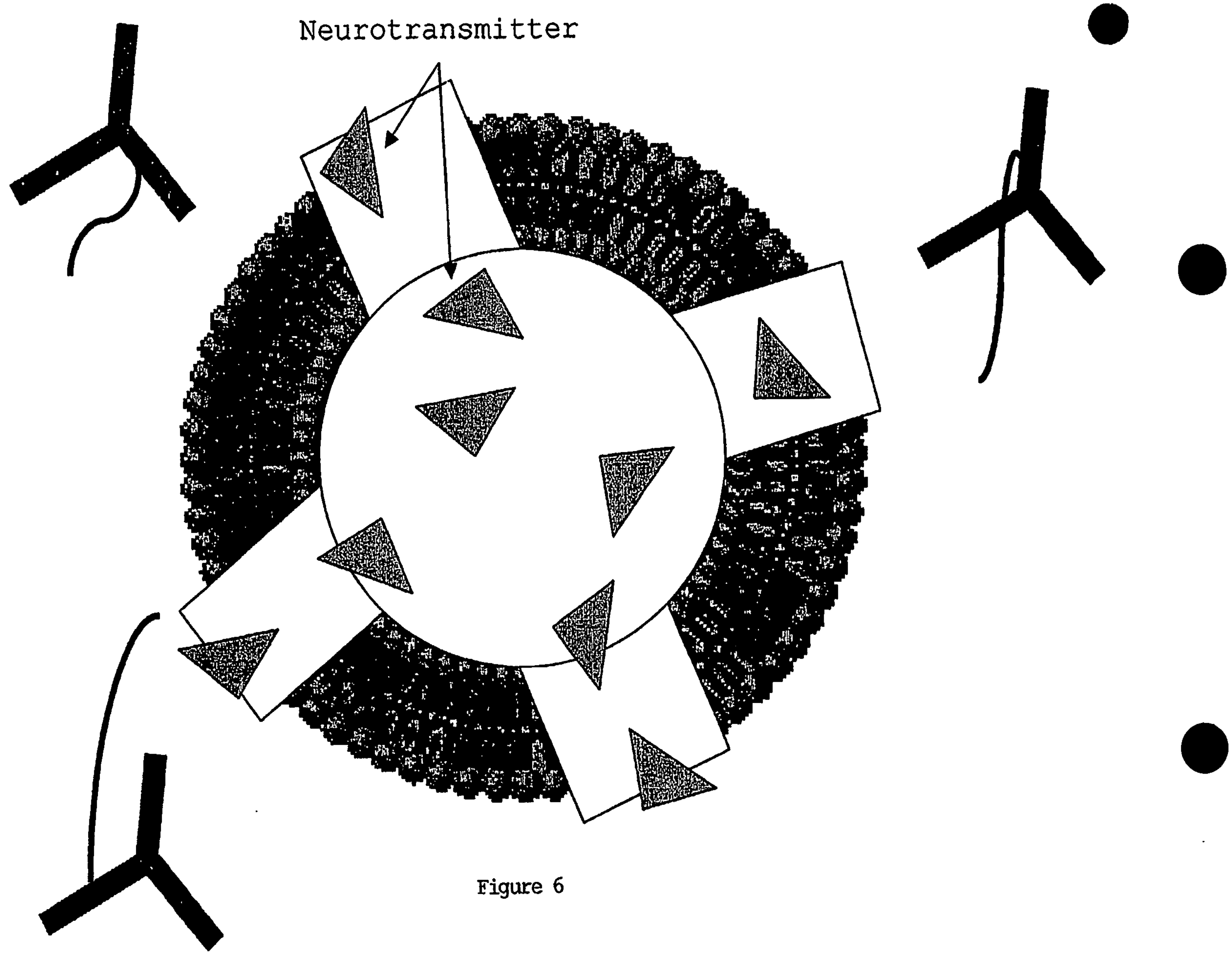


Figure 6

Non-invasive Neuronal Modulation

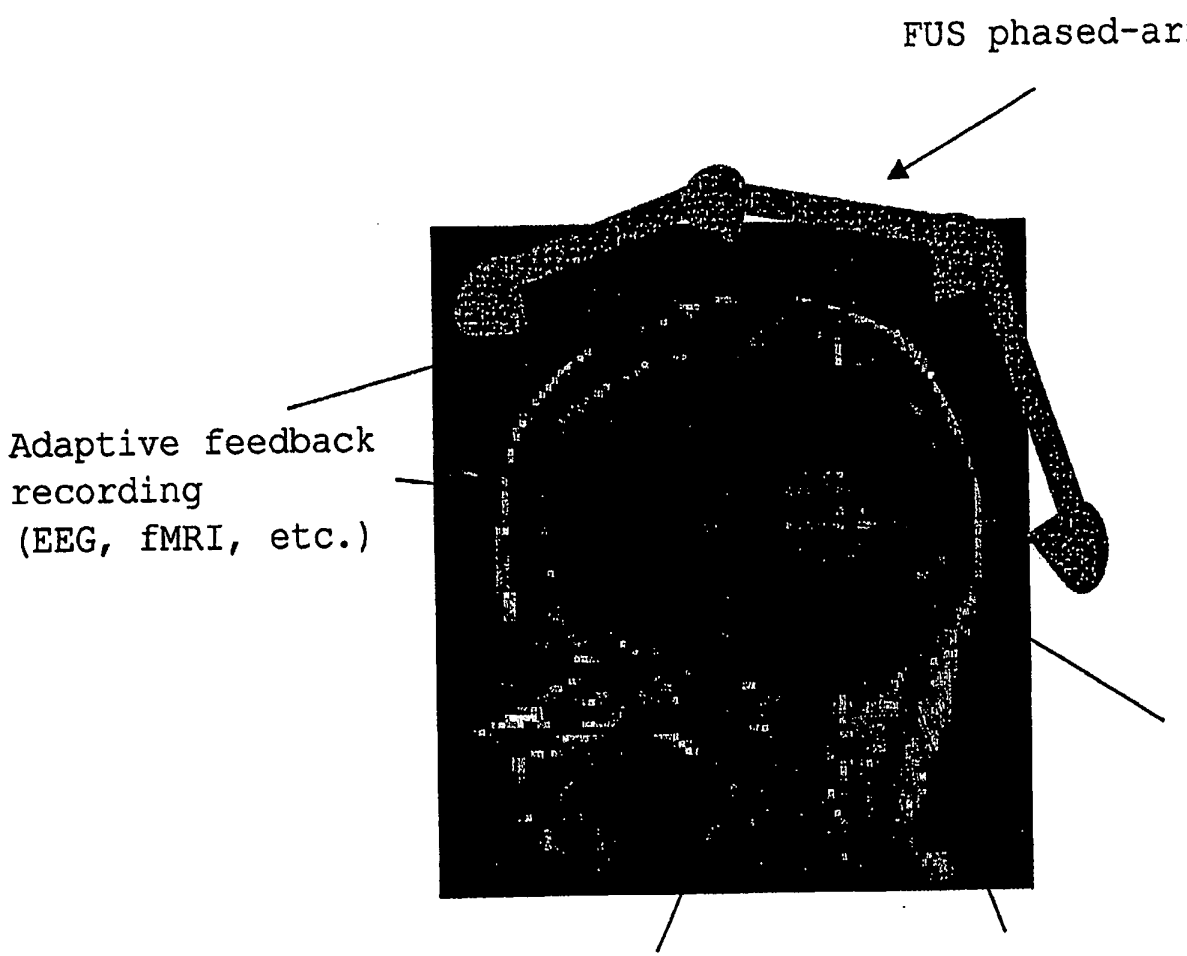


Figure 7